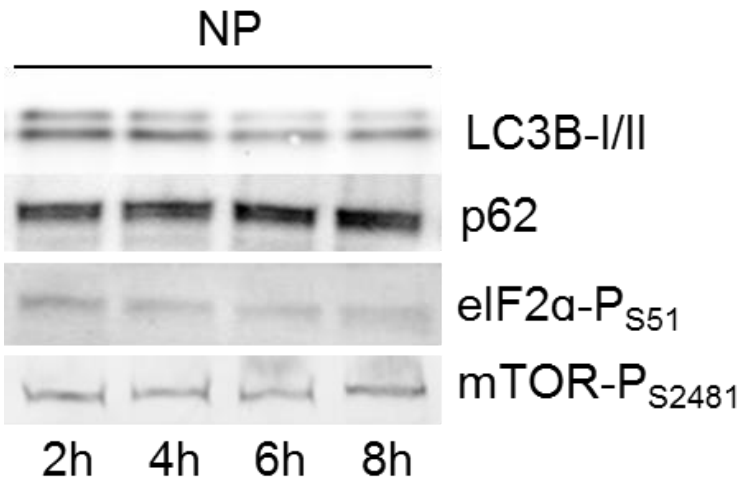
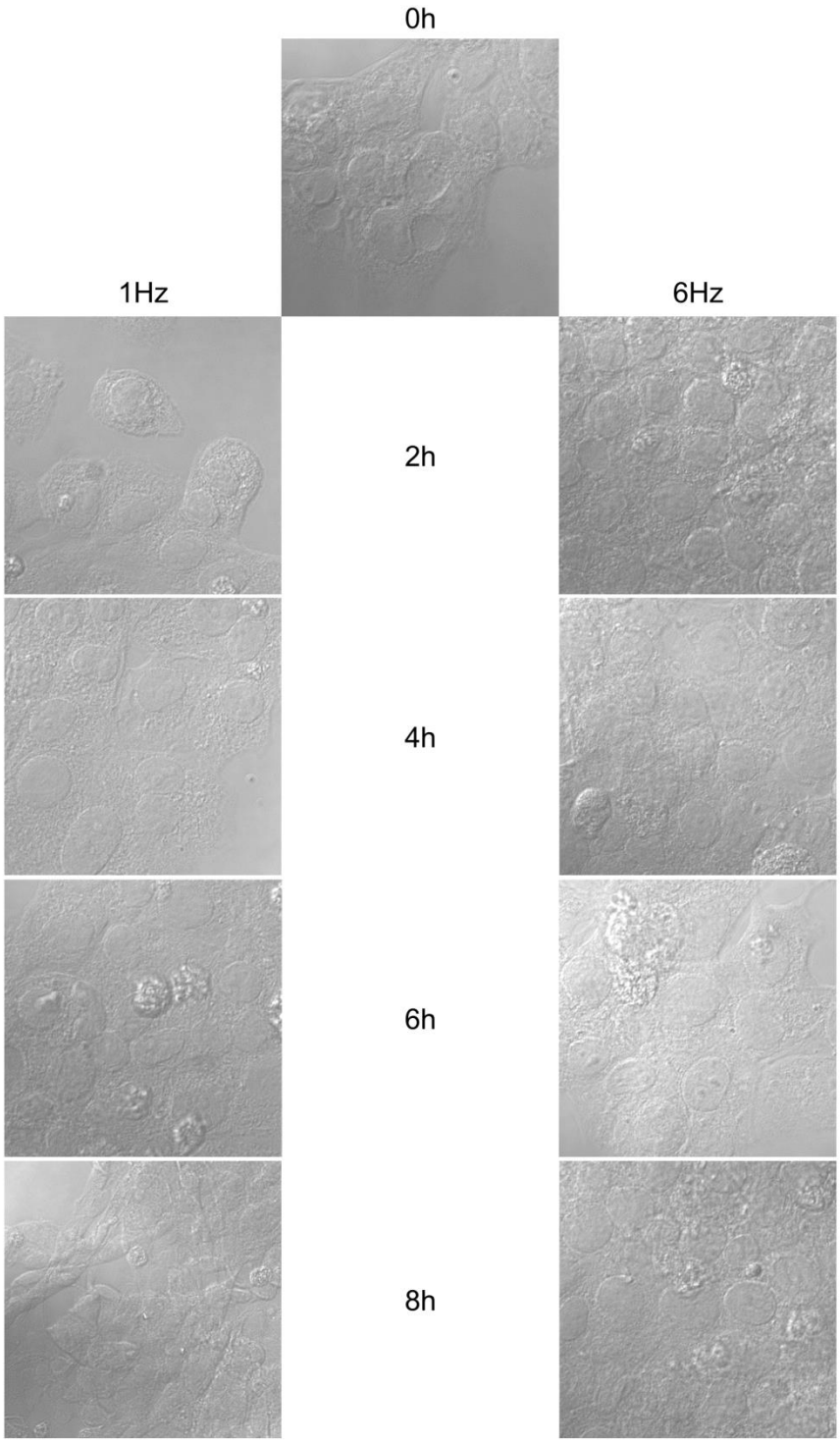
Supplemental Material

Figure S1. Normal pacing does not change protein expression.



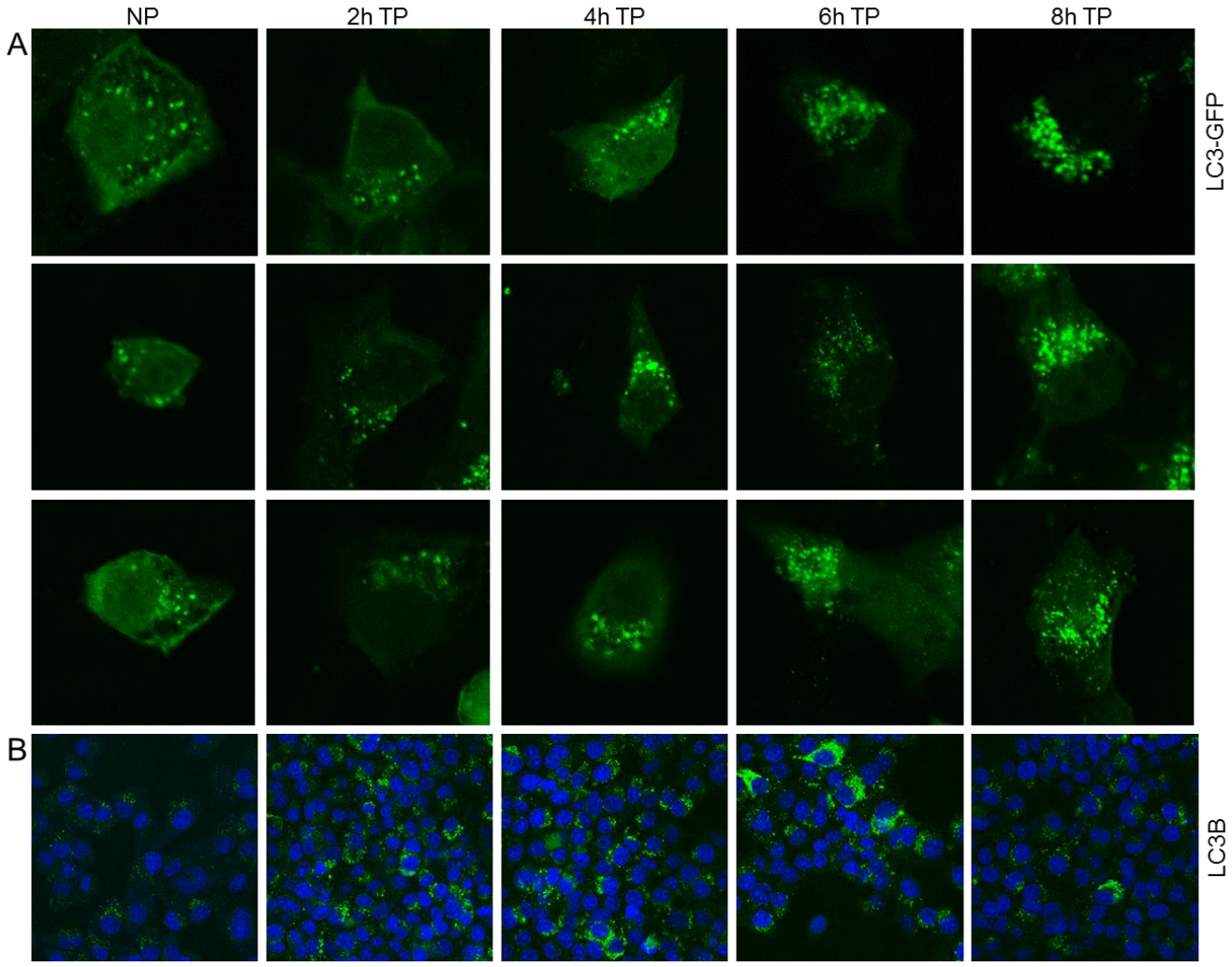
Representative Western blot showing LC3B-I/II, p62, eIF2α-P_{S51} and mTOR-P_{S2481} expression in normal paced HL-1 cardiomyocytes. Normal pacing does not change the protein expression levels of any of these proteins.

Figure S2. Normal pacing and tachypacing does not change cardiomyocyte morphology.



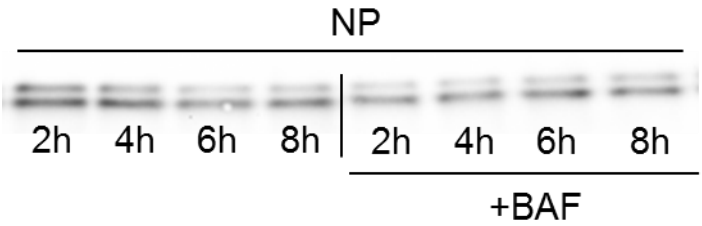
Bright field microscopic images showing the morphology of the HL-1 atrial cardiomyocytes after non-pacing (0 h), normal pacing (1 Hz) or tachypacing (6 Hz). No morphological changes were observed between the conditions as indicated.

Figure S3. Tachypacing induces autophagosome formation.



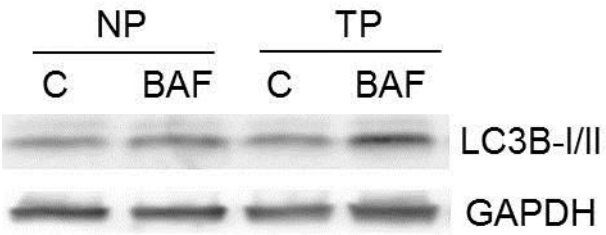
Confocal images of multiple tachypaced HL-1 cardiomyocytes, for the period as indicated, (**A**) transfected with LC3B-GFP or (**B**) endogenous LC3B visualized by immunostaining. Green puncta indicate autophagosomes.

Figure S4. LC3B-II levels are not increased during normal pacing after BAF treatment.



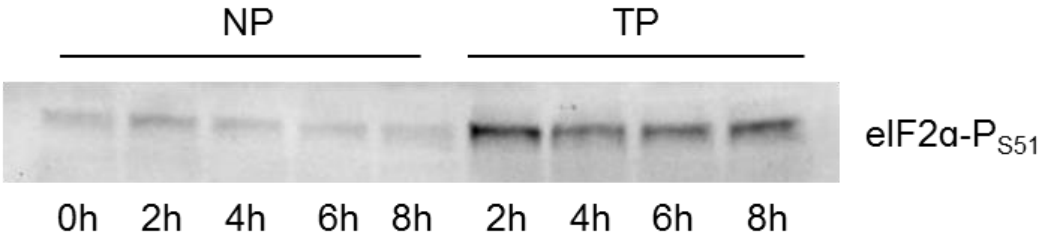
Representative Western blot showing LC3B-I/II expression in normal paced HL-1 cardiomyocytes with and without BAF treatment. BAF doesn't increase the LC3B-II levels during normal pacing.

Figure S5. BAF treatment further increases LC3B-II levels in tachypaced HL-1 cardiomyocytes.



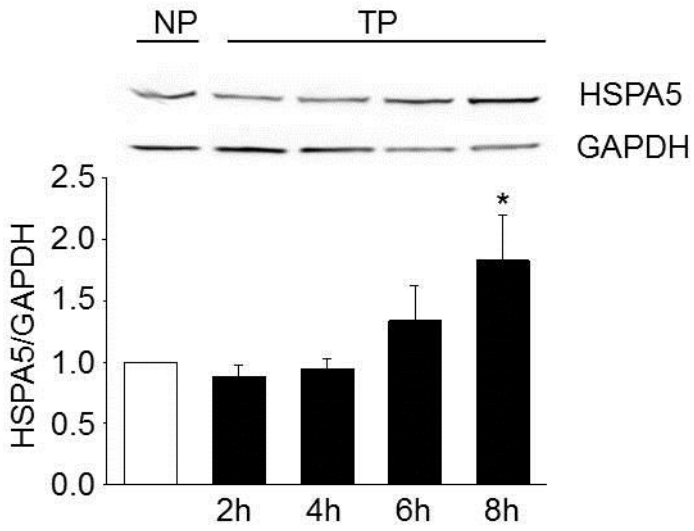
Representative Western blot showing LC3B-I/II expression in normal paced (NP) and 8 h tachypaced (TP) HL-1 cardiomyocytes with and without BAF treatment. BAF increases the LC3B-II levels after 8h tachypacing.

Figure S6. Normal pacing does not change eIF2α phosphorylation.



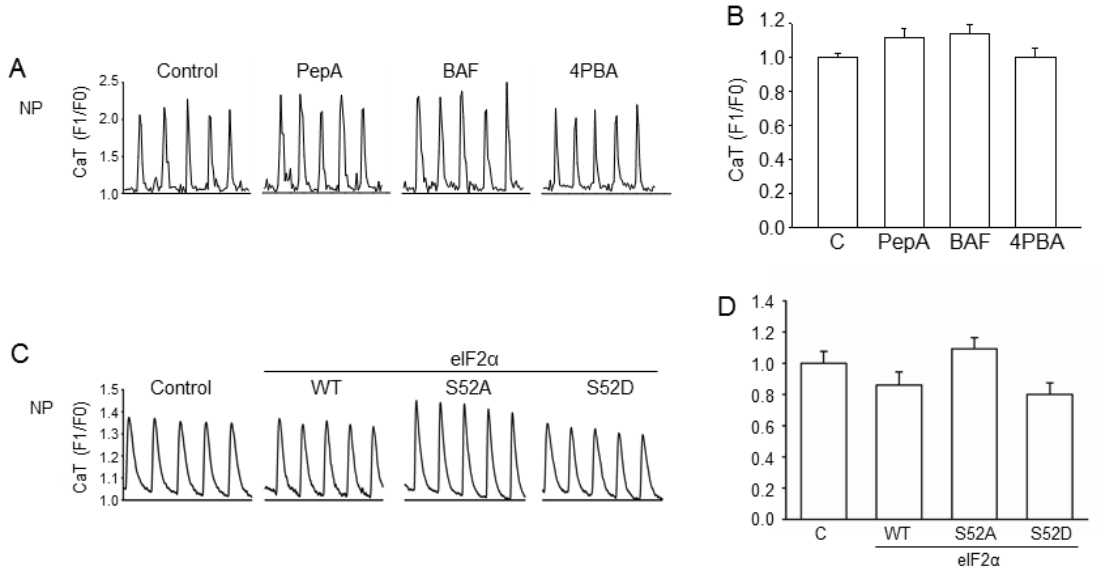
Representative Western blot showing eIF2α phosphorylation in normal paced (NP) and tachypaced (TP) HL-1 cardiomyocytes.

Figure S7. Tachypacing induces expression of the ER chaperone HSPA5.



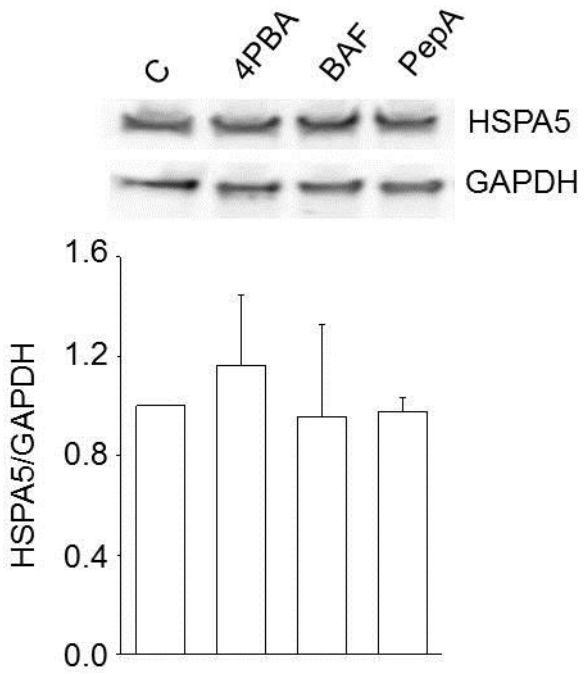
Top panel: representative Western blot showing HSPA5 and GAPDH expression in normal paced (NP) and tachypaced (TP) HL-1 cardiomyocytes. Bottom: quantified data revealing significant increase in HSPA5 levels in tachypaced HL-1 cardiomyocytes (N=3). *$P \leq 0.05$ vs NP.

Figures S8. The effect of pharmacological and genetic modulation of autophagy and ER stress in normal paced and tachypaced HL-1 cardiomyocytes.



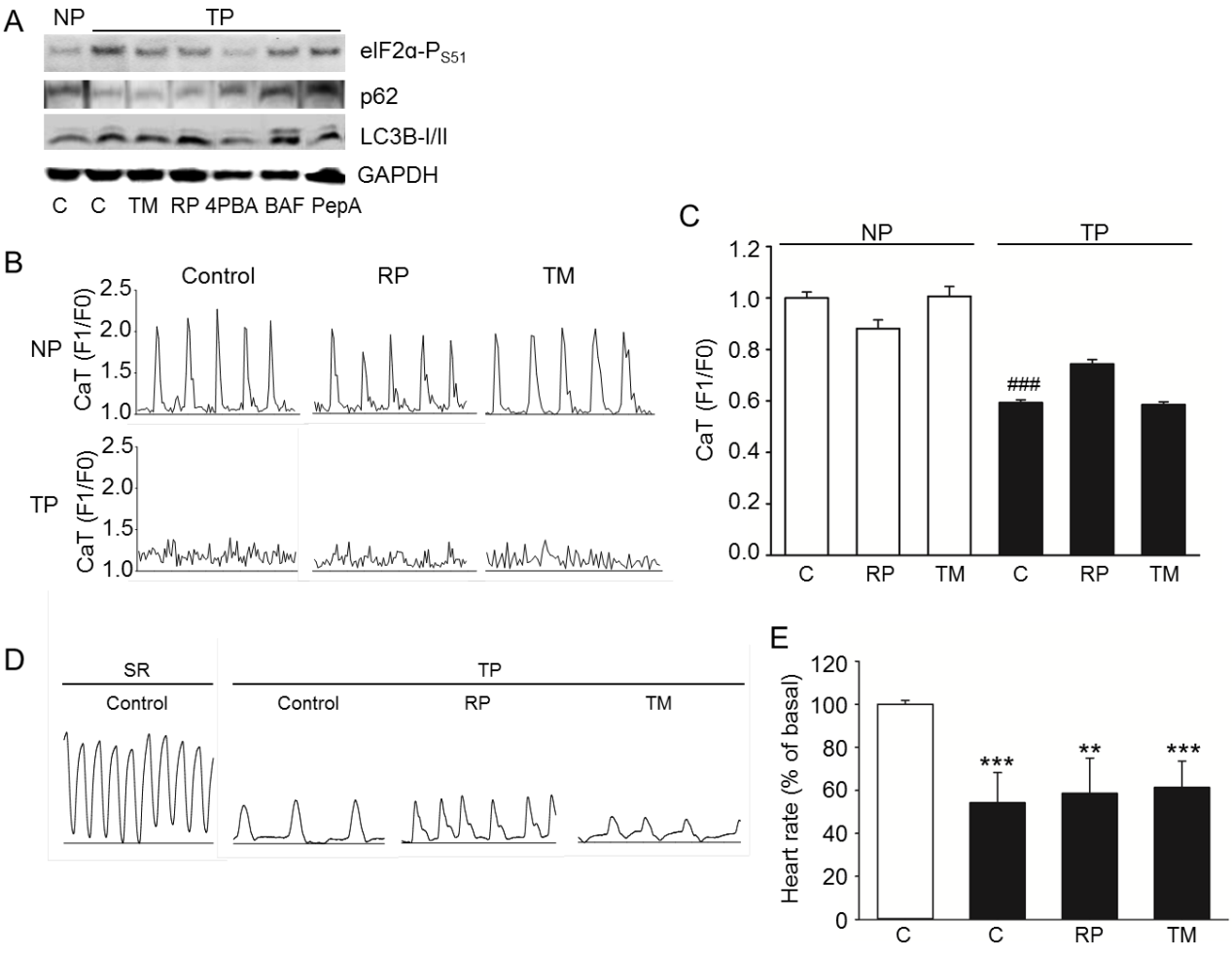
(**A**) Representative CaT (5 sec) of HL-1 cardiomyocytes after normal pacing (NP). HL-1 cardiomyocytes were pre-treated with the autophagy modulators PepA or BAF, or the chemical chaperone 4PBA, followed by normal and measurement of CaT. (**B**) Quantified CaT of HL-1 cardiomyocytes pretreated with pepstatin A, bafilomycin A1 or 4PBA and subjected to normal pacing, which does not change calcium transients (n/N=60/4). (**C**) Representative CaT (5 sec) of HL-1 cardiomyocytes transfected with empty plasmid (Control), eIF2α wild-type, non-phosphorylated (S52A) or phospho-mimetic (S52D) mutant and followed by NP. (**D**) Quantified CaT of HL-1 cardiomyocytes transiently transfected with empty plasmid (Control), eIF2α wild-type, non-phosphorylated (S52A) or phospho-mimetic (S52D) mutant and subjected to normal pacing, which does not change calcium transients (n/N=30/3).

Figure S9. Pharmacological modulation of autophagy in HL-1 cardiomyocytes does not change HSPA5 expression.



Top panel: representative Western blot showing HSPA5 and GAPDH levels in HL-1 cardiomyocytes pretreated with various autophagy modulators as indicated. Lower panel: quantified data showing no significant changes in HSPA5 levels for the conditions as indicated (N=3).

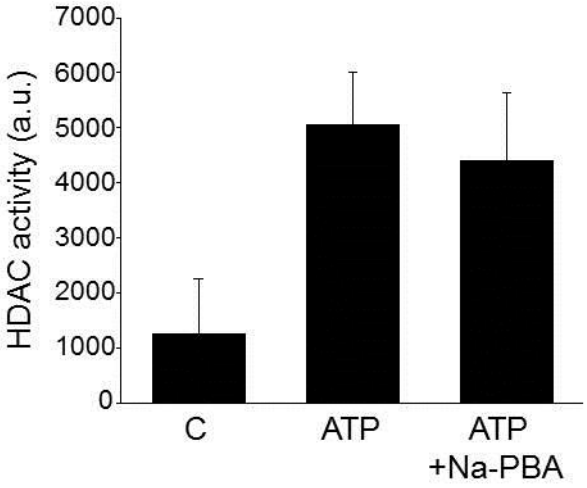
Figure S10. Activators of ER stress and autophagy do not protect against tachypacing-induced contractile dysfunction in HL-1 cardiomyocytes and *Drosophila*.



(**A**) Representative Western blot showing eIF2α-P$_{S51}$, p62, LC3B-I/II and GAPDH levels in HL-1 cardiomyocytes pretreated the ER stress-inducer tunicamycin (TM), the autophagy-inducer rapamycin (RP), autophagy inhibitors BAF and pepstatin A and the chemical chaperone 4PBA. RP and TM show no protection against tachypacing-induced changes in eIF2α-P$_{S51}$, p62 or LC3B-I/II expression. (**B**) Representative CaT of HL-1 cardiomyocytes after normal pacing (NP) or tachypacing (TP), pre-treated with TM or RP. (**C**) Quantified CaT amplitude of NP and TP HL-1 cardiomyocytes, each from groups as indicated (n/N=50/3). There is no significant decrease in CaT amplitude for either RP or TM at NP, due to the non-toxic concentrations applied.

Nevertheless, RP and TM did not protect against contractile dysfunction. (**D**) Representative heart wall contractions of *Drosophila* monitored before TP and after TP with DMSO (Control), TM or RP pretreatment. (**E**) Quantified data showing heart wall contraction rates from groups as indicated. RP and TM did not protect against contractile dysfunction. White bars represent normal paced (NP in HL-1 cardiomyocytes) or spontaneous heart rate (SR in *Drosophila*) and black bars represent tachypaced HL-1 cardiomyocytes or *Drosophila*. N=9 to 15 prepupae for each group. ***P*≤0.01, ****P*≤0.001 vs control SR, ###*P*≤0.001 vs control TP

Figure S11. 4PBA has no effect on HDAC activity in dogs.



Atrial tachypacing of dogs results in a borderline significant induction (*P*=0.06) of HDAC activity, which was not altered by 4PBA treatment (N= 7 dogs for each group).

Supplemental Video Legends:

Video S1. Time-lapse video shows CaT after 8 hours normal pacing (1Hz) of HL-1 cardiomyocytes. Images were acquired at 2 ms intervals.

Video S2. Time-lapse video shows CaT after 8 hours tachypacing (6Hz) of HL-1 cardiomyocytes. Images were acquired at 2 ms intervals.

Video S3. Time-lapse video shows CaT after 8 hours tachypacing (1Hz) of HL-1 cardiomyocytes pretreated with 4PBA. Images were acquired at 2 ms intervals.

Video S4. Time-lapse video shows CaT after 8 hours tachypacing (6Hz) of HL-1 cardiomyocytes pretreated with 4PBA. Images were acquired at 2 ms intervals.

Video S5. Time-lapse video shows CaT after 8 hours tachypacing (1Hz) of HL-1 cardiomyocytes transfected with empty plasmid. Images were acquired at 2 ms intervals.

Video S6. Time-lapse video shows CaT after 8 hours tachypacing (6Hz) of HL-1 cardiomyocytes transfected with empty plasmid. Images were acquired at 2 ms intervals.

Video S7. Time-lapse video shows CaT after 8 hours tachypacing (1Hz) of HL-1 cardiomyocytes transfected with HSPA5 construct. Images were acquired at 2 ms intervals.

Video S8. Time-lapse video shows CaT after 8 hours tachypacing (6Hz) of HL-1 cardiomyocytes transfected with HSPA5 construct. Images were acquired at 2 ms intervals.

Video S9. Time-lapse video shows CaT after 8 hours tachypacing (1Hz) of HL-1 cardiomyocytes pretreated with pepstatin A. Images were acquired at 2 ms intervals.

Video S10. Time-lapse video shows CaT after 8 hours tachypacing (6Hz) of HL-1 cardiomyocytes pretreated with pepstatin A. Images were acquired at 2 ms intervals.

Video S11. Time-lapse video shows CaT after 8 hours tachypacing (1Hz) of HL-1 cardiomyocytes pretreated with BAF. Images were acquired at 2 ms intervals.

Video S12. Time-lapse video shows CaT after 8 hours tachypacing (6Hz) of HL-1 cardiomyocytes pretreated with BAF. Images were acquired at 2 ms intervals.

Video S13. Time-lapse video shows heart wall contractions of a prepupae of a W1118 genetic background before tachypacing, and after tachypacing (5Hz, **Video S14**).

Video S15. Time-lapse video shows heart wall contractions of a prepupae of a W1118 genetic background pretreated with BAF before tachypacing, and after tachypacing (5Hz, **Video S16)**.

Video S17. Time-lapse video shows heart wall contractions of a prepupae of a W1118 genetic background pretreated with 4PBA before tachypacing, and after tachypacing (5Hz, **Video S18**).